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EFFECT OF SODIUM TRANSPORT AND VASOPRESSIN ON THE OXIDATION OF PALMITIC ACID BY THE TOAD BLADDER

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SUMMARY

1. The object of this investigation was to examine some factors influencing the rate of oxidation of palmitate by the toad bladder, with special reference to the effects of vasopressin and changes in active sodium transport.

2. Palmitic acid is oxidized to CO_2 by the toad bladder. This rate of oxidation is increased when the ratio moles of palmitate/moles of albumin in the incubation medium is higher, and also when glucose is present.

3. Inhibition of active sodium transport, either by using sodium-free media or adding ouabain reduces the rate of palmitate oxidation.

4. Vasopressin increases palmitate oxidation in the presence of sodium, not in its absence.

INTRODUCTION

Vasopressin stimulates active sodium transport by the isolated toad bladder and in addition accelerates the oxidation of a number of substrates, including glucose, glycogen and pyruvate[1-3]. The alterations in substrate metabolism induced by the hormone occur only in the presence of sodium in the bathing medium and thus do not reflect a direct action of vasopressin on substrate. Since fatty acids are an important energy source in many tissues and vasopressin is known to affect lipolysis in adipose tissue[4,5], the effect of vasopressin on the oxidation of labelled palmitate by the toad bladder in the presence and absence of sodium transport was examined. GOODFRIEND AND KIRKPATRICK[3] had previously reported acceleration of palmitate oxidation in the toad bladder by vasopressin when sodium was present in the bathing medium, but had not determined whether these changes were related to sodium transport *per se* or reflected a direct effect of vasopressin on fatty acid oxidation.

METHODS

Toads (*Bufo marinus*) were kept at room temperature on San-i-cel moistened with tap water. They were killed by pithing and their urinary bladders dissected free and placed in normal Ringer's solution. Each half bladder was cut open with scissors to ensure free access of the incubation medium to both surfaces of the tissue.

In most studies one half-bladder was used as the experimental preparation and the other half as the control. Incubations were performed in 25-ml erlenmeyer flasks containing 3 ml of incubation medium. All incubations were done at room temperature (24°) in a metabolic shaker. Bladders were pre-incubated for two 30-min periods in normal Ringer's solution unless otherwise stated.

Apart from experiments in which the tissue was pre-loaded with [1-^{14}C]-palmitate bladders were incubated in Ringer's solution containing [1-^{14}C]palmitate for 1 h in capped erlenmeyer flasks. At the end of this incubation CO_2 was released with sulphuric acid and trapped in hyamine. Each flask was then shaken for a further hour before transferring the hyamine containing CO_2 into 15-ml volumes of scintillation fluid. Samples were counted in a Packard Tri-Carb liquid scintillation counter.

Each half-bladder was removed from the incubation flask into a weighed bottle, dried overnight at 90° and reweighed to obtain the dry weight.

The amphibian Ringer's solution contained 90 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgSO_4$, 0.5 mM KH_2PO_4, and 5.5 mM glucose. In sodium-free Ringer's solution the Na^+ was replaced by (hydroxymethyl) aminomethane (Tris) and the pH adjusted by bubbling with CO_2. All solutions were bubbled for 10 min before use with 97 % O_2 and 3 % CO_2 (pH 7.70).

Palmitate solutions were prepared by adding potassium palmitate to fatty acid-free bovine albumin[6] solutions. The total palmitate concentrations are given for each experiment and the molar ratio of palmitate to albumin or $\bar{\nu}$ is also given.

The scintillation fluid contained toluene 818 ml/l, Triton X-100 182 ml/l, PPO (2,5-diphenyloxazole) 5.5 g/l, and dimethyl POPOP (1,4-bis-2-[4-methyl-5-phenyl-oxazolyl]benzene) 100 mg/l.

Arginine vasopressin from bovine pituitaries was kindly given to us by Dr. P. J. BENTLEY.

Results were analyzed using Student's "t" test using half-bladders from the same toad as paired samples.

RESULTS

Effect of varying palmitate: albumin ratios ($\bar{\nu}$) on the rate of oxidation of [1-^{14}C]palmitate (Table I)

It is apparent that exogenous palmitate is oxidised to CO_2 by the toad bladder. Oxidation is greater at $\bar{\nu}$ 4, *i.e.* in the presence of more free fatty acid, as might be

TABLE I

EFFECTS OF GLUCOSE AND CONCENTRATION OF FREE [^{14}C]PALMITATE ON $^{14}CO_2$ PRODUCTION
Number of pairs: 8.

Glucose concn.	*$^{14}CO_2$ production (μmoles × 10^4/mg per h)*			*P*
	$\bar{\nu}^* = 1$	$\bar{\nu}^* = 4$	$\bar{\nu}$ 4 − $\bar{\nu}$ 1 (± S.E.)	
Zero	0.81	3.07	+2.26 ± 0.86	< 0.05
5.5 mM	1.46	5.35	+3.89 ± 0.57	< 0.001
Δ due to glucose	+0.66 ± 0.13	+2.23 ± 0.86		
P	<0.005	<0.05		

* $\bar{\nu}$ = molar ratio of palmitate to albumin.

expected. In this experiment each toad bladder was divided into 4 segments, one quarter from each bladder was incubated at $\bar{v}$ 1 and $\bar{v}$ 4 both in the presence and absence of glucose. Of interest is the observation also noted in Table I that $^{14}CO_2$ produced from palmitate is greater in the presence of glucose than in its absence.

Effect of vasopressin on [1-^{14}C]palmitate oxidation (Table II)

It can be seen that $^{14}CO_2$ production from palmitate is accelerated by vasopressin in the presence of sodium (Expt. A). Since this effect may have been a consequence of facilitation of entry of palmitate into the cell by vasopressin, Expt. B was performed. In this the bladders were pre-incubated without hormone in tracer amounts of [1-^{14}C]palmitate (spec. activ. 54.1 mC/mmole). At the end of this period adherent palmitate was removed as far as possible by washing in fresh palmitate-free Ringer's solution, then incubating in 2 % albumin solution for 1 h. After the hour in 2 % albumin the bladders were placed in CO_2-collecting flasks with and without vasopressin. Under these circumstances $^{14}CO_2$ production was, as before, accelerated by the hormone.

TABLE II

EFFECT OF VASOPRESSIN ON $^{14}CO_2$ PRODUCTION

Expt. A: Half-bladders were incubated in sodium Ringer's containing [1-^{14}C]palmitate ($\bar{v}$ = 4) with and without vasopressin for 1 h during which $^{14}CO_2$ was collected. *Expt. B*: Half-bladders were incubated in sodium Ringer's containing [1-^{14}C]palmitate ($\bar{v}$ = 3) for 1 h, washed in fresh Ringer's, transferred to sodium Ringer's containing bovine serum albumin (20 mg/ml) for 1 h, then transferred to fresh medium with or without vasopressin and incubated for 1 h during which $^{14}CO_2$ was collected. *Expt. C*: This experiment was similar to Expt. A except that all sodium in the medium was replaced by Tris and $\bar{v}$ = 3.

Expt.	*Number of pairs*	*Palmitate* ($\mu M \times 10^3$)	*Vasopressin* (*munits/ml*)	$^{14}CO_2$ *production* (μ*moles* $\times$ 10^5/*mg per h*)		*P*
				Control	*Δ due to vasopressin* (± *S.E.*)	
A	14	630	20	33.2	+16.8 ± 5.1	<0.01
B	16	10	15	0.82	+0.27 ± 0.064	<0.005
C	17	0.49	20	0.29	+0.013 ± 0.063	>0.10 n.s.

TABLE III

EFFECT OF ABOLISHING SODIUM TRANSPORT ON $^{14}CO_2$ PRODUCTION

Expt.	*Experimental conditions*	*Palmitate concn.* ($\mu M \times 10^4$)	$^{14}CO_2$ *production* (μ*moles* $\times$ 10^5/*mg per h*)	
			Mean	*Mean effect* ± *S.E.*
D	Sodium Ringer's	12.0	1.89	
	Tris replacing sodium (n = 6)	12.0	0.45	−1.44 ± 0.49 ($P < 0.01$)
E	Sodium Ringer's	4.9	0.432	
	plus ouabain $2 \cdot 10^{-4}$ M (n = 8)	4.9	0.200	−0.232 ± 0.084 ($P < 0.05$)

Effect of abolishing active sodium transport on $^{14}CO_2$ production (Table III)

From the results of Expts. D and E it is evident that abolition of active sodium transport either by omitting sodium from the incubation medium or by adding $2 \cdot 10^{-4}$ M ouabain significantly diminishes $^{14}CO_2$ production. In addition vasopressin had no effect on the rate of $^{14}CO_2$ production in the absence of sodium (Expt. C, Table II).

DISCUSSION

The results of the present studies establish that the increased oxidation of palmitate by the toad bladder due to vasopressin is dependent on the presence of active sodium transport and is not a reflection of a direct effect of the hormone on fatty acid metabolism. The results of Expt. B are consistent with the view that endogenous fatty acids may provide energy for sodium transport, though this has not yet been established with certainty. On the basis of preliminary studies it appears unlikely that the intracellular pool of non-esterified [1-^{14}C]palmitate alone is adequate to account for the amount of $^{14}CO_2$ produced in these studies. Thus it is probable that energy for active sodium transport is derived not only from oxidation of carbohydrate as had previously been established, but also from oxidation of fatty acids, both free and esterified.

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REFERENCES

1 A. LEAF AND E. DEMPSEY, *J. Biol. Chem.*, 235 (1960) 2160.
2 R. H. MAFFLY AND I. S. EDELMAN, *J. Gen. Physiol.*, 46 (1963) 733.
3 T. GOODFRIEND AND J. KIRKPATRICK, *Endocrinol.*, 72 (1963) 742.
4 D. RUDMAN, S. J. BROWN AND M. F. MALKIN, *Endocrinol.*, 72 (1963) 527.
5 M. VAUGHAN, *Am. J. Physiol.*, 207 (1964) 1166.
6 R. F. CHEN, *J. Biol. Chem.*, 242 (1967) 173.